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Modulation of therapeutic antibody effector functions by glycosylation engineering: influence of Golgi enzyme localization domain and co-expression of heterologous beta1, 4-N-acetylglucosaminyltransferase III and Golgi alpha-mannosidase II.

Ferrara C, Brünker P, Suter T, Moser S, Püntener U, Umaña P.

GLYCART biotechnology AG, Wagistrasse 18, CH-8952 Schlieren, Switzerland.

The effector functions elicited by IgG antibodies strongly depend on the carbohydrate moiety linked to the Fc region of the protein. Therefore several approaches have been developed to rationally manipulate these glycans and improve the biological functions of the antibody. Overexpression of recombinant beta1,4-N-acetylglucosaminyltransferase III (GnT-III) in production cell lines leads to antibodies enriched in bisected oligosaccharides. Moreover, GnT-III overexpression leads to increases in non-fucosylated and hybrid oligosaccharides. Such antibody glycovariants have increased antibody-dependent cellular cytotoxicity (ADCC). To explore a further variable besides overexpression of GnT-III, we exchanged the localization domain of GnT-III with that of other Golgi-resident enzymes. Our results indicate that chimeric GnT-III can compete even more efficiently against the endogenous core alpha1,6-fucosyltransferase (alpha1,6-FucT) and Golgi alpha-mannosidase II (ManII) leading to higher proportions of bisected non-fucosylated hybrid glycans ("Glyco-1" antibody). The co-expression of GnT-III and ManII led to a similar degree of non-fucosylation as that obtained for Glyco-1, but the majority of the oligosaccharides linked to this antibody ("Glyco-2") are of the complex type. These glycovariants feature strongly increased ADCC activity compared to the unmodified antibody, while Glyco-1 (hybrid-rich) features reduced complement-dependent cytotoxicity (CDC) compared to Glyco-2 or unmodified antibody. We show that apart from GnT-III overexpression, engineering of GnT-III localization is a versatile tool to modulate the biological activities of antibodies relevant for their therapeutic application. (c) 2006 Wiley Periodicals, Inc.

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Expression of GnTIII in a recombinant anti-CD20 CHO production cell line: Expression of antibodies with altered glycoforms leads to an increase in ADCC through higher affinity for FC gamma RIII. [Biotechnol Bioeng. 2001]

Inhibition of hybrid- and complex-type glycosylation reveals the presence of the GlcNAc transferase I-independent fucosylation pathway [Glycobiology. 2006]

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